

**AMENDMENTS TO THE CLAIMS**

1-171. Canceled.

172. (Previously Presented) A method of synthesizing a polypeptide array, wherein said array comprises at least two polypeptides, which differ in composition, immobilized on a substrate, said method comprising:

- (a) contacting a surface of the substrate with a first protected amino acid wherein said first protected amino acid becomes selectively coupled to a functional group selected from the group consisting of:
  - (i) a functional group attached to the substrate;
  - (ii) a functional group attached to a linker that is attached to the substrate;
  - (iii) a functional group attached to an amino acid that is coupled to the substrate;
  - (iv) a functional group attached to an amino acid that is coupled to a linker that is attached to the substrate;
  - (v) a functional group attached to a nascent polypeptide that is coupled to a linker that is attached to the substrate;
  - (vi) a functional group attached to a nascent polypeptide coupled to the substrate; and
  - (vii) combinations thereof,wherein the functional group is in a first selectively activated region of said surface, and wherein a protecting group has been removed from said first selectively activated region of the surface without removing protecting groups from other positionally defined locations of the substrate;
- (b) contacting said surface with a second protected amino acid wherein said second protected amino acid becomes selectively coupled to a functional group selected from the group consisting of:
  - (i) a functional group attached to the substrate;
  - (ii) a functional group attached to a linker that is attached to the substrate;
  - (iii) a functional group attached to an amino acid that is coupled to the substrate;

- (iv) a functional group attached to an amino acid that is coupled to a linker that is attached to the substrate;
  - (v) a functional group attached to a nascent polypeptide that is coupled to a linker that is attached to the substrate;
  - (vi) a functional group attached to a nascent polypeptide coupled to the substrate; and
  - (vii) combinations thereof,
- wherein the functional group is in a second selectively activated region of said surface, and wherein a protecting group has been removed from said second selectively activated region of the surface without removing protecting groups from other positionally defined locations of the substrate; and,
- (c) repeating the above steps until at least two polypeptides, which differ in composition, are formed at positionally defined locations on said substrate surface.

173. (Previously Presented) The method as recited in claim 172, wherein contacting said surface with said second protected amino acid is accomplished without physical segregation of said surface.
174. (Previously Presented) The method as recited in claim 172, wherein said at least two different polypeptides each occupy an area on said substrate of less than about  $1 \text{ cm}^2$  to about  $1 \times 10^{-5} \text{ cm}^2$ .
175. (Previously Presented) The method as recited in claim 174, wherein said at least two different polypeptides each occupy an area on said substrate of less than about  $1 \times 10^{-1} \text{ cm}^2$  to about  $1 \times 10^{-4} \text{ cm}^2$ .
176. (Previously Presented) The method as recited in claim 175, wherein said at least two different polypeptides each occupy an area on said substrate of less than about  $1 \times 10^{-2} \text{ cm}^2$  to about  $1 \times 10^{-3} \text{ cm}^2$ .

177. (Previously Presented) The method as recited in claim 172, repeating said steps above until said at least two different polypeptides exceed a density of about 400 different polypeptides/cm<sup>2</sup>.
178. (Previously Presented) The method as recited in claim 172, repeating said steps above until said at least two different polypeptides exceed a density of about 1000 different polypeptides/cm<sup>2</sup>.
179. (Previously Presented) The method as recited in claims 172, wherein said method produces a substrate that contains more than 100 different polypeptides per cm<sup>2</sup>.
180. (Previously Presented) The method as recited in claim 172, wherein said method produces a substrate that contains more than 1,000 different polypeptides per cm<sup>2</sup>.
181. (Previously Presented) The method as recited in claim 172, wherein said method produces a substrate that contains more than 10,000 different polypeptides per cm<sup>2</sup>.
182. (Previously Presented) The method as recited in claim 172, wherein said method produces a substrate that contains more than 100,000 different polypeptides per cm<sup>2</sup>.
183. (Previously Presented) A method for synthesizing polypeptides on a substrate, said method comprising:
- a) providing a substrate wherein said substrate comprises immobilized polypeptide molecules, said polypeptide molecules coupled to a removable protecting group;
  - b) removing said protecting group from said polypeptide molecules in a first positionally defined location of said substrate without removing said protecting groups from a second positionally defined location of said substrate; and
  - c) contacting said substrate with a first amino acid to couple said first amino acid to said polypeptide molecules in said first positionally defined location, said first amino acid having an amino acid protecting group thereon, forming a first polypeptide on said substrate in said positionally defined location that is different in composition from a polypeptide in said second positionally defined location.

184. (Previously Presented) The method as recited in claim 183, wherein said step of removing is an irradiation step.
185. (Previously Presented) The method as recited in claim 184, wherein said irradiation step is a step of masking a light source with a mask placed between said light source and said substrate, said mask comprising first transparent regions and second opaque regions, said transparent regions transmitting light from said source to at least said first positionally defined location, and said opaque regions blocking light from said source to at least said second positionally defined location.
186. (Previously Presented) The method as recited in claim 183, wherein said first and second positionally defined location each have total areas less than about  $1 \text{ cm}^2$ .
187. (Previously Presented) The method as recited in claim 184, wherein said irradiation steps are conducted with a monochromatic light.
188. (Previously Presented) The method as recited in claim 184, wherein said irradiation steps and contacting are repeated so as to synthesize  $10^3$  different polypeptides on said substrate.
189. (Previously Presented) The method as recited in claim 184, wherein the irradiation step for a first positionally defined location is a step of irradiating half of a positionally defined location of said substrate irradiated in a prior synthesis step, and not irradiating half of said positionally defined location irradiated in a prior synthesis step.
190. (Previously Presented) The method as recited in claim 183, wherein said steps a) and b) are repeated to synthesize more than 1,000 different polypeptides on different synthesis locations of said substrate, each of said different polypeptides occupying an area of less than about  $10^{-2} \text{ cm}^2$  to about  $1 \times 10^{-5} \text{ cm}^2$ .

191. (Previously Presented) The method as recited in claim 190, wherein said steps a) and b) are repeated to synthesize more than 1,000 different polypeptides on different synthesis locations of said substrate, each of said different polypeptides occupying an area of less than about  $10^{-2} \text{ cm}^2$  to about  $1 \times 10^{-4} \text{ cm}^2$ .
192. (Previously Presented) The method as recited in claim 191, wherein said steps a) and b) are repeated to synthesize more than 1,000 different polypeptides on different synthesis locations of said substrate, each of said different polypeptides occupying an area of less than about  $10^{-2} \text{ cm}^2$  to about  $1 \times 10^{-3} \text{ cm}^2$ .
193. (Previously Presented) A method of synthesizing polypeptides, said method comprising:
- a) generating a pattern of light and dark areas by selectively irradiating at least a first area of a surface of a substrate, said surface comprising immobilized amino acids on said surface, said amino acids coupled to a photoremovable protective group, without irradiating at least a second area of said surface, to remove said protective group from said amino acids in said first area;
  - b) simultaneously contacting said first area and said second area of said surface with a first amino acid to couple said first amino acid to said immobilized amino acids in said first area, and not in said second area, said first amino acid having said photoremovable protective group;
  - c) generating another pattern of light and dark areas by selectively irradiating with light at least a part of said first area of said surface and at least a part of said second area to remove said protective group in said at least a part of said first area and said at least a part of said second area;
  - d) simultaneously contacting said first area and said second area of said surface with a second amino acid to couple said second amino acid to said immobilized amino acids in at least a part of said first area and at least a part of said second area; and
  - e) performing additional irradiating and amino acid contacting and coupling steps so that a matrix array of at least 100 polypeptides, which differ in composition, is formed on said surface, each different polypeptide synthesized in an area of less than  $0.1 \text{ cm}^2$ , whereby said different polypeptides have sequences and locations

on said surface defined by the patterns of light and dark areas formed during the irradiating steps and the amino acids coupled in said contacting steps.

194. (Previously Presented) The method as recited in claim 193, wherein said substrate is selected from the group consisting of Langmuir Blodgett film, glass, germanium, silicon, (poly)tetrafluorethylene, polystyrene, gallium arsenide, gallium phosphide, silicon oxide, silicon nitride, and combinations thereof.
195. (Previously Presented) The method as recited in claim 193, wherein said protective group is one or more protective groups selected from the group consisting of 6-nitroveratryloxycarbonyl, 2-nitrobenzyloxy carbonyl, dimethyl dimethoxybenzyloxy carbonyl, 5-bromo-7-nitroindoliny, o-hydroxyalpha-methyl cinnamoyl, and 2-oxymethylene anthriquinone.
196. (Previously Presented) The method as recited in claim 193, wherein each different polypeptide synthesized is in an area of less than about  $0.1 \text{ cm}^2$  to about  $1 \times 10^{-5} \text{ cm}^2$ .
197. (Previously Presented) The method as recited in claim 196, wherein each different polypeptide synthesized is in an area of less than about  $1 \times 10^{-1} \text{ cm}^2$  to about  $1 \times 10^{-4} \text{ cm}^2$ .
198. (Previously Presented) The method as recited in claim 197, wherein each different polypeptide synthesized is in an area of less than about  $1 \times 10^{-2} \text{ cm}^2$  to about  $1 \times 10^{-3} \text{ cm}^2$ .
199. (Previously Presented) The method as recited in claim 193, wherein said matrix array is at least 400 different polypeptides/ $\text{cm}^2$ .
200. (Previously Presented) The method as recited in claim 193, wherein said matrix array is at least 1000 different polypeptides/ $\text{cm}^2$ .
201. (Previously Presented) The method as recited in claim 193, wherein said method produces a substrate that contains more than, 1,000 different polypeptides per  $0.1 \text{ cm}^2$ .

202. (Previously Presented) The method as recited in claim 193, wherein said method produces a substrate that contains more than 10,000 different polypeptides per  $0.1 \text{ cm}^2$ .
203. (Previously Presented) The method as recited in claim 193, wherein said method produces a substrate that contains more than 100,000 different polypeptides per  $0.1 \text{ cm}^2$ .
204. (Previously Presented) The method as recited in claim 193, wherein the irradiating step c) further comprises:
- i) placing a mask adjacent to said substrate, said mask having substantially transparent regions and substantially opaque regions at a wavelength of light; and
  - ii) illuminating said mask with a light source, said light source producing at least said wavelength of light, said mask permitting illumination of half of said substrate which was illuminated and half of said substrate which was not illuminated in said step a).
205. (Previously Presented) The method as recited in claim 204, wherein said additional steps are performed so as to synthesize  $10^3$  different polypeptides in  $10^3$  respective positionally defined locations on said substrate.
206. (Previously Presented) The method as recited in claim 204, wherein said additional steps are performed so as to synthesize  $10^6$  different polypeptides in  $10^6$  respective positionally defined locations on said substrate.
207. (Previously Presented) The method of claim 204, wherein said additional steps are performed so that at least 1,000 different polypeptides are synthesized on said surface, and each different polypeptide is contained within an area less than about  $1 \times 10^{-3} \text{ cm}^2$ .
208. (Previously Presented) The method of claim 193, wherein said immobilized amino acids are attached to said surface by a linker selected from the group consisting of aryl acetylene and ethylene glycol.

209. (Previously Presented) The method of claim 193, wherein said immobilized amino acids are attached to said surface by polyethylene glycol.
210. (New) A method for forming a plurality of different polypeptides occupying positionally defined locations on a substrate by sequential addition of amino acids to said substrate, comprising:
- (a) providing a substrate having a surface with a localized area occupied by a protected amino acid;
  - (b) deprotecting the protected amino acid in a positionally defined location within the localized area to produce a deprotected amino acid;
  - (c) reacting the deprotected amino acid with a protected amino acid, whereby the protected amino acid attaches to the deprotected amino acid to produce a protected polypeptide; and
  - (d) repeating steps (b) and (c) at least once wherein the positionally defined location in step (b) comprises a fraction of a positionally defined location in a previous deprotecting step (b) to produce an array of different polypeptides occupying different positionally defined locations within the localized area.
211. (New) The method as recited in claim 210, wherein contacting said surface with said protected amino acid of step (c) is accomplished without physical segregation of said surface.
212. (New) The method as recited in claim 210, wherein said different polypeptides each occupy an area on said substrate of less than about  $1 \text{ cm}^2$  to about  $1 \times 10^{-5} \text{ cm}^2$ .
213. (New) The method as recited in claim 210, repeating said steps above until said different polypeptides exceed a density of about 400 different polypeptides/ $\text{cm}^2$ .
214. (New) The method as recited in claim 210, wherein the protected amino acid of step (a) is:



- (i) an amino acid that is coupled to the substrate;
- (ii) an amino acid that is coupled to a linker that is attached to the substrate;
- (iii) an amino acid in a nascent polypeptide that is coupled to a linker that is attached to the substrate;
- (iv) an amino acid in a nascent polypeptide coupled to the substrate; or
- (v) a combination thereof.